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## ANTINOCICEPTIVE ACTIVITY OF THE AQUEOUS EXTRACT OF *BAUHINIA CHEILANTHA* (BONG.) STEUD. (LEGUMINOSAE: CAESALPINIOIDEAE)


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### ABSTRACT

*Bauhinia cheilantha* (Leguminosae: Caesalpinioideae) is a common plant of the Brasilian Caatinga widely used in folk medicine as an analgesic. In order to verify this effect pharmacologically, the antinociceptive activity of the plant was evaluated through the administration of its aqueous extract in mice. The extract was administered orally (400 mg/kg) 60 minutes before a writhing test, and was found to reduce nociception by 54.4%. The effects of formalin (1%) were also reduced by the extract at all doses. Naloxone (5 mg/kg, i.p.) and caffeine (10 mg/kg, i.p) reverted the effect of the extract. In a hot plate test, the extract (100mg, 200mg and 400 mg/kg) increased latency time by 39.8%, 30.7% and 32.8%, respectively. There was no acute toxicity in doses up to 3g/kg. The aqueous extract of the *B. cheilantha* bark revealed antinociceptive activity in all the models tested, effects that are possibly associated with the opioid and adenosine systems.



**Keywords:** *Bauhinia cheilanta*, antinociceptive, aqueous extract.



### RESUMO

*Bauhinia cheilantha* (Leguminosae-Caesalpinioideae) é uma planta comum da caatinga amplamente utilizada na medicina popular como analgésica. Para verificar farmacologicamente este efeito, a atividade antinociceptiva desta planta foi verificada através do extrato aquoso da entre-casca, administrado em camundongos. O extrato (400 mg/kg), administrado por via oral 60 minutos antes do teste reduziu a nocicepção em 54,4%. Os efeitos da formalina (1%) também foram reduzidos pelo extrato em todas as doses. Naloxona (5 mg/kg, i.p.) e cafeína (10 mg/kg, i.p) também reverteram este efeito. No teste da placa quente o extrato de *B. cheilantha* nas doses 100mg, 200mg e 400 mg/kg, aumentou o tempo de latência em 39.8%, 30.7% e 32.8%, respectivamente. Não houve toxicidade aguda até a dose de 3g/Kg. O extrato aquoso da entre-casca de *B. cheilantha* mostrou atividade antinociceptiva nos modelos testados, efeito possivelmente associado com os sistemas opióide e adenosina.



**Palavras-chave:** *Bauhinia cheilanta*, antinociceptivo, extrato aquoso.


### INTRODUCTION

Many plants of the Brasilian flora are used in local folk medicine, but very few have been pharmacologicaly validated. Among the unstudied majority is the legume *Bauhinia cheilantha* (Bong.) Steud., a small tree (average height 5 m), which is relatively common in the semi-arid Brasilian Caatinga. Known locally as *mororó*, the extract of its bark is used as an analgesic, but there are no pharmacological data on its properties.

A variety of pharmacological properties have been reported for species of the genus *Bauhinia*. For example, the seeds of *Bauhinia monandra* affect haemagglutination positively, although this effect is eliminated by autoclaving (Abreu et al, 1990; Penate et


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al, 1988), and the leaves of *Bauhinia tarapotensis* have antioxidant properties (Braca et al., 2001). Other species of the genus *Bauhinia* have been reported to have properties related to the treatment of malaria and hypoglycemia, and the regulation of thyroid hormones (Kittakoop et al., 2000; Lemus et al., 1999; Pepato et al., 2002; Panda & Kar, 1999).

Several chemical compounds have been purified from different species of *Bauhinia*, including lectins, racemosol, and demethylracemosol. (Silva & Filho, 2002; Allen et al., 1980, Kittakoop et al., 2000). The medicinal uses of *B. cheilantha* have been reported in the ethnobotanical literature (eg. Andrade-Lima, 1988), but there have been no pharmacological studies of the analgeic properties of this plant, a lack of information which motivated the present study.

## MATERIAL AND METHODS

**Plant material**: Bark of *B. cheilantha* was collected during the wet season in the village of Santa Rosa do Ermírio, Sergipe (09°45'S, 37°40'W). The species was identified by the biologist Gilvane V. Souza, and the voucher specimen (number 007490) is deposited at the herbarium of the Departamento de Biologia, Universidade Federal de Sergipe, Brasil.

**Preparation of aqueous extract (AE)**: The bark was dried at 40°C in a forced air oven (Marconi MA 037) for 48 h and triturated in a mill to obtain a powder. This powder was added to distilled water (1:5 w/v) at 75°C and infused for 30 min in order to constitute the aqueous extract. After infusion, the AE was filtered and freeze dried in a VirTis bench top freeze dryer, yielding a brown powder (8%), which was diluted and used in the pharmacological tests. For the experiments, the extract was reconstituted in water at three different concentrations – 100 mg/mL (AE100), 200 mg/mL (AE200) and 400 mg/mL (AE400), which were administered orally to the mice 60 minutes before each experiment.

**Animals**: Male and female Swiss mice (20-35 g) were used as test animals. The animals were maintained in plastic cages, with food and water *ad libitum*, but were fasted for 12 hours prior to the oral administration of test substances. All experiments were performed in accordance with current guidelines for the care of laboratory animals and ethical guidelines for investigations of experimental pain in conscious animals.

**Preparation of drugs:** Drugs used in the experiments were diluted to an injection volume of 0.1 mL/10g of animal weight, except when defined in the text. Acetic acid 0.6% (Merck), morphine hydrochloride (Sigma), formalin 1% (Baker), naloxone hydrochloride (Sigma), and caffeine (Sigma) were all diluted in water. Indomethacin (Sigma) was diluted in water/0.1 N NaOH (pH = 8). Indomethacin, morphine, naloxone and caffeine were injected 15 minutes before tests.

**Acute toxicity**: Acute toxicity of the plant was verified in three groups of mice (n = 5/ne group (n = 5) received distilled water; the others received the aqueous extract in increasing doses (1, 2, and 3 g/kg; p.o.). The mortality was observed during 48 hours.

**Pharmacological tests**

Antinociceptive activity was verified using the writhing test induced by acetic acid 0.6% (Koster et al. 1959), the formalin 1% test (Dubuisson et al., 1977; Hunskaar, 1986) and the hot plate test (Eddy & Leimbach, 1953).

*Writhing test*: Six groups of mice (n = 9) were tested. All animals received acetic acid 0.6% (0.1 mL/ 10g; i.p.), and AE100, AE200 and AE400 was administered p.o. to three groups 1 h before the nociceptive agent. One control group received distilled water instead of the aqueous extract. Morphine (2.5 mg/kg, i.p.) and indomethacin (10 mg/kg, i.p.) were used as standards in the remaining groups. Ten minutes after the acid was administrated, the number of writhes was

recorded during a period of 20 min.

*Hot plate test:* The experiment started 60 min (time zero) after p.o. administration of AE100, AE200 and AE400. The mice (n = 9) were placed on an aluminum plate heated to 55±0.5ºC and the time elapsed to the moment when they licked their hind paws was recorded (hereafter referred to as latency). This procedure was repeated at times 0, 15, 30 and 60 min. In order to avoid damage to the paws of the animals, time standing on the plate was limited to 30 seconds. Morphine (5 mg/ kg, i.p.) was used as the reference drug.

*Formalin test:* In a group of nine mice, AE100, AE200 and AE400 was administered p.o., and after 60 min., 0.02 mL of 1% formalin was administered to the subplantar of the left posterior paw. The time that each mouse spent licking its paw was recorded. Control animals received distilled water, p.o. Morphine was used as a standard test drug (7.5 mg/kg, i.p.). The reaction to pain was measured 0-5 min (1st phase) and 20-25 min (2nd phase) after administration of the stimulus. In order to confirm the possible participation of the opioid system, naloxone (5 mg/kg; i.p.) was administered with AE100 to a second group (n = 8). Morphine combined with naloxone (7.5 mg/kg, i.p. and 5 mg/kg; i.p., respectively) was administered to a third group 15 minutes prior to the injection of formalin. We also administered caffeine (10 mg/kg, i.p.) associated with AE100 to verify the influence of the adenosine system on the effect of the aqueous extract.

**Statistical analysis**: Results of the hot plate test were analysed using two-factor parametric ANOVA, in which the variable was the latency and the two factors being simultaneously verified were the time interval of the four experiments and the drugs (extract and morphine dosage, and the control). The Tukey test for multiple comparisons of the means was applied to all F values with $p<0.05$. The results of the writhing and formalin tests were analysed using the Kruskal-Wallis non-parametric one-way ANOVA (samples do not come from normal population and the variances are heterogenous), with the Nemenyi test for multiple comparisons of the medians (Zar, 1996).

Percentage inhibition = (1-Vt/Vc) x 100, where Vt and Vc represent the number of writhes or time spent licking the paw for the treated and control groups, respectively. The percentage increase in latency = (1-(Xc/Xt)x 100), where Xc and Xt are the average latency for control and treated groups, respectively.

## RESULTS AND DISCUSSION

The aqueous extract of *B. cheilantha* reduced significantly the frequency of writhing induced by acetic acid (Kruskal-Wallis: $F_{0,05;5;47} = 32.4$; $p<0.0005$), with the number of events reduced by up to 54.4%, in comparison with the control, at 400 mg/kg (Nemenyi: $q_{0,05;\infty;6} = 5.1$; $p<0.05$, Table 1). Positive results for the writhing test do not necessarily means that the extract has an analgesic effect, however, as this test is sensitive not only to analgesics, but also to substances such as muscle relaxants, antihistamines, monoamine oxidase inhibitors, adrenergic blockers, and neuroleptics (Chernov *et al*., 1967; Hendershot & Forsaith, 1959; Loux *et al*., 1978; Pearl *et al*., 1968). The formalin and hot plate tests were employed in order to confirm the analgesic properties of the extract.

The hot plate model involves a supraspinal response to thermal stimuli. Drugs with supraspinal action affect this response, especially at temperatures of 55ºC or more (Ankier, 1974; Magalini *et al*., 1979). The results indicate no statistical difference in mean latency among the experiments at time zero, 15, 30 and 60 min (ANOVA: $F_{0.05(1)3,140} = 0.956$; $p>0,05$; Table 2) and no interaction of the experiments with drugs (including control) affecting latency means ($F_{0.05(1)12,140} = 0.735$, $p>0.05$). These results indicate homogeneity in the mean latency of control, morphine, and extract groups in all four experiments, but the most important general finding was the significant differences among drugs (including control), independently of time (ANOVA: $F_{0.05(1)4,140} = 27.8$; $p< 0.0005$).

The accentuated significance of this result may be due primarily to the morphine effect, but we were particularly interested in verifying the contribution of the extract to the significance of the variance and which dose affected the result. As no significant differences were found among experiments, the grand mean of each extract dose was compared to the grand mean of the control. The results indicate that all doses of the extract were significantly different from control, with an increase of 39.8% (AE100), 30.7% (AE200) and 32.8% (AE400) in latency of the response to the administration of the extract. (Tukey: AE100, AE200, and AE400 vs. control group, respectively: $q_{0.05,140,5} = 6.74$; $p<0.05$; $q_{0.05,140,5} = 4.51$; $p<0.05$; $q_{0.05,140,5} = 4.9$; $p<0.05$; Table 2). This response suggests that, like morphine, the aqueous extract interferes with the central pain induced mechanism, reducing its nociceptive response to thermal stimuli.

The aqueous extract of *B. cheilantha* altered the paw licking time significantly in both phases of the test (Kruskal-Wallis: $F_{0.05(1)4;39} = 15.3$; $p<0.0005$; $F_{0.05(1)4;39} = 13.7$; $p<0.0005$; respectively). In the first phase, AE100 reduced paw licking time significantly (Nemenyi: $q_{0.05,\infty,5} = 4.1$; $p<0.05$). In the second phase, all doses inhibited the formalin effect (Nemenyi: $q_{0.05,\infty,5,} = 5.3$; $p<0.05$; $q_{0.05,\infty,5} = 4.2$; $p<0.05$; $q_{0.05,\infty,5} = 4.2$; $p<0.05$). Table 3 shows the percentage inhibition of the pain reaction produced by morphine and the plant's aqueous extract.

Naloxone, a non-selective opioid receptor antagonist, was used to elucidate the possible mechanisms of the *B. cheilantha* extract (Magalini *et al*., 1979). In the first phase, Kruskal-Wallis revealed no significant differences among the following groups: control, morphine plus naloxone, and AE100 plus naloxone (Kruskal-Wallis: $F_{0.05(1)2;20} = 2.2$; $p>0.10$, Table 4), which suggests that the effect of the aqueous extract may involve an opioid system. However, when comparing the control, AE100, and AE100 plus caffeine groups, significant differences were found in both phases of the test (Kruskal-Wallis: $F_{0.05(1)2,20} = 13.2$;

Table 1. Effects of control, AE100, AE200, and AE400, indomethacin (10 mg/kg), and morphine (2.5 mg/kg) on the frequency of writhing induced by acetic acid 0.6%.

| Group | Dose (mg/kg) | Median | % |
|---|---|---|---|
| Control | - | 27 | - |
| Morphine | 2.5 | 3* | 69.2 |
| Indomethacin | 10 | 2* | 79.8 |
| AE | 100 | 18 | 17.2 |
| AE | 200 | 14 | 41.9 |
| AE | 400 | 6* | 54.4 |

n = 9
* $p<0.05$ compared to control, Nemenyi test after Kruskal-Wallis.
% = percentage of writhes inhibition scores.

Table 2. Effects of *B. cheilantha* aqueous extract (AE) and morphine on latency time in the hot plate test.

| Groups | Dose (mg/kg) | Latency time (Mean±SEM) | | | | Grand Mean | % |
|---|---|---|---|---|---|---|---|
| | | 0 min | 15 min | 30 min | 60 min | | |
| Control | - | 13.11±1.02 | 13.25±1.29 | 9.55±1.15 | 8.73±1.33 | 11.16 | - |
| Morphine | 5 | 29.09±0.91 | 25.66±2.17 | 27.52±1.26 | 25.59±1.83 | 26.96* | 58.6 |
| AE | 100 | 18.45±2.22 | 14.93±1.67 | 19.83±2.88 | 20.92±2.66 | 18.53* | 39.8 |
| AE | 200 | 15.78±1.56 | 14.71±2.51 | 17.5±2.70 | 16.4±2.57 | 16.1* | 30.7 |
| AE | 400 | 18.45±1.92 | 14.87±1.56 | 16.38±2.3 | 16.75±2.31 | 16.61* | 32.8 |

n = 9 for each group at each time interval.
* p<0,05, compared to control, Tukey test after two way ANOVA.
%, percentage of latency time increase.
SEM, standard error of the mean.

$p<0.0005$; $F_{0.05(1)2,20} = 82.7$; $p<0.0005$, respectively). In the first phase, caffeine reverted the AE effect (Nemenyi: $q_{0.05\ \infty;5} = 1.8$; $p>0.05$; Table 5), which indicates participation of the adenosine system in the analgesic effect. Adenosine may interact with four subtypes of receptors ($A_1$, $A_{2a}$, $A_{2b}$ and $A_3$), but, as caffeine is a non-selective antagonist, further studies will be necessary to determine how these subtypes are involved in the response.

In order to evaluate the acute toxicity of *B. cheilantha*, the mice were submmited to increasing doses of the extract. No deaths ocurred, even at the highest dose (3 mg/kg), indicating low toxicity, as reported by Lorke (1993).

Table 3. Effect of *B. cheilantha* aqueous extract (AE, 100, 200, and 400 mg/kg) and morphine (7.5 mg/kg) in the 1% formalin test.

| Groups | Dose (mg/kg) | 1st phase | | 2nd phase | |
|---|---|---|---|---|---|
| | | Median | % | Median | % |
| Control | - | 35 | - | 21 | - |
| Morphine | 7.5 | 10* | 77.4 | 0* | 64.0 |
| AE | 100 | 20* | 53.9 | 0* | 57.4 |
| AE | 200 | 24 | 17.9 | 0* | 46.1 |
| AE | 400 | 29 | -3.7 | 0* | 46.2 |

n = 9 for each group at each time interval
* $p<0.05$ compared to control, Nemenyi test after Kruskal-Wallis.
% = inhibition percentage of pain reaction scores.

Table 4. Effect of naloxone (5 mg/kg, i.p.) on morphine (10 mg/kg, p.o.) and AE100 in the formalin test.

| Groups | 1st phase | | 2nd phase | |
|---|---|---|---|---|
| | Median | % | Median | % |
| Control | 54.5 | - | 29.5 | - |
| Morphine + Naloxone | 55.5 | -10.9 | 18 | 21.2 |
| AE100 + Naloxone | 47.5 | 38.2 | 0* | 73.3 |

n = 8 for each group at each tim e interval.
*$p<0.05$ compared to control, Nemenyi test after Kruskal-Wallis .
% = inhibition percentage of pain reaction scores

Table 5. Effects of caffeine on AE100 in the formalin test.

| Groups | 1st phase | | 2nd phase | |
|---|---|---|---|---|
| | Median | % | Median | % |
| Control | 53.5 | - | 27.0 | - |
| AE100 | 23.5* | 68.7 | 0.0* | 56.1 |
| AE100 + Caffeine | 37.0 | 25.1 | 0.0* | 61.0 |

n = 8 for each group at each time interval.
*$p<0.05$ compared to control, Nemenyi test after Kruskal-Wallis.
% = inhibition percentage of pain reaction scores.

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